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Microsatellite DNA Markers Detects 95% of Chromosome 22q11 Deletions

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Cono-truncal cardiac malformations account for some 50% of congenital heart defects in newborn infants. Recently, hemizygosity for chromosome 22q11.2 was reported in patients with the DiGeorge/Velo-cardio-facial syndromes (DGS/VCFS) and causally related disorders. We have explored the potential use of microsatellite DNA markers for rapid detection of 22q11 deletions in 19 newborn infants referred for cono-truncal heart malformations with associated DGS/VCFS anomalies. A failure of parental inheritance was documented in 84.2% of cases (16/19). PCR-based genotyping using microsatellite DNA markers located within the commonly deleted region allowed us either to confirm or reject a 22q11 microdeletion in 94.3% of cases (18/19) within 24 hours. This test is now currently performed in the infants referred to us for a cono-truncal heart malformation as a first intention screening for 22q11 microdeletion. Am. J. Med. Genet. 68:182–184, 1997

KEY WORDS: chromosome 22q11.2 microdeletions; congenital heart disease; conotruncal heart defect; microsatellite DNA markers

INTRODUCTION

Cono-truncal cardiac malformations account for some 50% of congenital heart defects in newborn infants. Recently, hemizygosity for 22q11.2 was reported in patients with the DiGeorge syndrome (DGS) and related disorders, including the velo-cardio-facial syndrome (VCFS), the cono-truncal anomaly face syndrome, and the Cayler cardio-facial syndrome [Burn et al., 1993; Driscoll et al., 1992; Gianotti et al., 1994; Scambler et al., 1991]. These disorders most frequently occur de novo and cause impaired development of the cardiac outflow tract resulting in cono-truncal malformations [Moerman et al., 1980].

We have explored the potential use of microsatellite DNA markers for rapid detection of 22q11 deletions in newborn infants referred for cono-truncal heart malformations. Here, we show that three highly informative microsatellite DNA markers located within the region commonly deleted in DGS/VCFS [Demczuk et al., 1995; Marineau et al., 1992; Morrow et al., 1995] detected deletions in a large proportion of cases and therefore were used as reliable tools both for diagnosing 22q11 deletions and for counselling at-risk families.

METHODS

From November 1994 to July 1995, 19 patients were included in the study. Clinical criteria for inclusion were i) a cono-truncal heart malformation documented by ultrasound study in the neonatal period (this included truncus arteriosus communis, interrupted aortic arch, tetralogy of Fallot (with or without pulmonary valve atresia, or with absent pulmonary valve), ventricular septal defect with malalignment of the conal septum, and complex cyanotic cardiac defects with malposition of the great arteries); and ii) minor facial anomalies with hypertelorism, and/or telecanthus, shortness of palpebral fissures, unusual nose, small mouth, malformed ears, or cleft palate. No history of familial congenital heart disease was noted. Serum calcium was sampled at least twice in the first 2 weeks of life. Thymus, kidney, and brain were assessed by ultrasound study.

EDTA-blood samples were collected from the affected individuals (2–3 ml) and their parents (10 ml). White blood cells (30 ml) were boiled in NaOH 10 mM, NaCl 200 mM and SDS 0.05% (500 ml) for 10 minutes. PCR amplifications were performed in a total volume of 25 ml, containing 1 ml white blood cells, 50 pmol of each primer, 1.25 mM dNTPs, and 0.4 unit Taq DNA polymerase in 10 mM Tris HCl, pH9, 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X100, and 0.01% gelatin. Amplification conditions were i) initial denaturation at 96°C for 10 min: ii) 30 cycles of 96°C for 40 seconds, 55°C for 30 seconds, 72°C for 40 seconds; and iii) a final exten-

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sion of 10 minutes at 72°C. PCR products were mixed with 5 ml formamide loading dye (95% formamide, 10 mM NaOH, 0.5% bromophenol blue, and 0.05% xylene cyanol). The samples were denatured at 95°C for 5 minutes, directly loaded onto a 6% polyacrylamide-urea sequencing gel and electrophoresed. The gels were blotted onto nylon membranes (Appligene), labeled by chemiluminescence according to the manufacturer's instructions (ECL direct nucleic acid labeling and detection systems; Amersham Life Science), and exposed to X-ray film for 10 minutes.

The three polymorphic CA-repeats location is as follow cen-TUPLE1-D22S941-D22S944-COMT-D22S264-ZNF74 [Morrow et al., 1995].

RESULTS

A failure of parental inheritance of either maternal (11 cases) or paternal origin (5 cases) at loci D22S264, D22S941, and D22S944 could be detected in 84.2% of cases (16/19; Fig. 1). These observations were not caused by false paternity, as genotype analysis using several markers from other chromosomes showed the expected codominant inheritance (likelihood of correct inheritance > 99%, not shown). In 2/19 cases, the index case was heterozygous at the three loci tested excluding a parental non-contribution at these loci. The fluorescent in situ hybridization (FISH) study technique confirmed the absence of microdeletion in these patients (probe Sc11.1 D22S139, not shown) [Desmaze et al. 1993]. In 1/19 patient, loss of heterozygosity could not be demonstrated, owing to a lack of informativity at the three loci. This patient exhibited only one allele at each locus and the FISH study confirmed the 22q11 microdeletion in this patient. Taken together, PCR-based genotyping using microsatellite DNA markers mapping to the minimal DGS/VCFS region allowed us to either confirm or reject a microdeletion at this locus in 94.3% of the cases in our series (Table I).

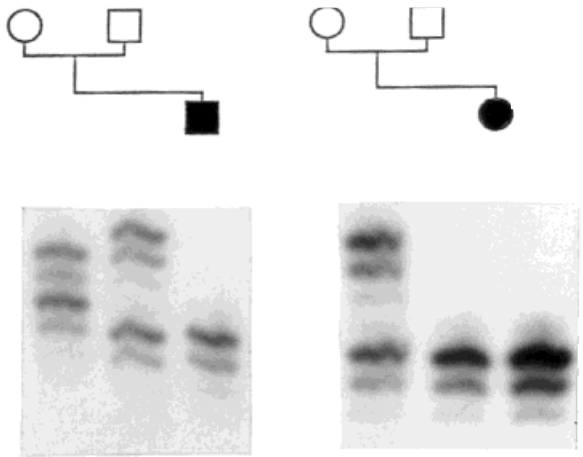

Fig. 1. Hemizygosity at locus D22S944 in sporadic DiGeorge syndrome. **A:** The index case failed to inherit a maternal allele at locus D22S944. **B:** A non-informative family.

TABLE I. Clinical Presentation and Molecular Studies at Loci D22S264, D22S941, and D22S944 in DGS/VCFS*

Patient	Cono-truncal heart defect	Minor anomalies	Hypocalcemia (<1.8 mmol/l)	Thymic aplasia or hypoplasia	Parental non-contribution		
					D22S264	D22S944	D22S941
1	IAA	DGS/VCFS typical appearance	+	+	M	NI	M
2	IAA	DGS/VCFS typical appearance	+	+	P	NI	P
3	IAA	DGS/VCFS typical appearance	+	+	NI	M	M
4	IAA	DGS/VCFS typical appearance	+	+	Heterozygous	P	P
5	IAA	DGS/VCFS typical appearance	+	+	M	M	M
6	IAA	Short palpebral fissures, prominent nasal tip	–	+	M	M	NI
7	TOF-PA	DGS/VCFS typical appearance	+	+	NI	P	P
8	TOF-PA	DGS/VCFS typical appearance	+	+	M	M	M
9	TOF-PA	DGS/VCFS typical appearance	+	+	M	M	M
10	TOF-PA	DGS/VCFS typical appearance	+	+	P	NI	P
11	TOF-PA	DGS/VCFS typical appearance	+	+	NI	NI	NI
12	TOF-PA	DGS/VCFS + cleft palate	+	+	M	M	M
13	TOF-PA	Epicanthic fold, long slender fingers	–	+	M	NI	M
14	TO	Broad face, bulbous nose tip, depressed nasal bridge	–	–	Heterozygous	Heterozygous	Heterozygous
15	TAC	DGS/VCFS typical appearance	+	–	NI	P	P
16	TAC	DGS/VCFS + cleft palate	+	+	NI	M	M
17	TAC	DGS/VCFS typical appearance	–	+	M	M	M
18	VSD	Broad nasal tip, long slender fingers	+	+	M	NI	M
19	Absent PV	Hypertelorism, small mouth	–	–	Heterozygous	Heterozygous	Heterozygous
Total		14/19	14/19	16/19	11/19	11/19	13/19

*IAA, interrupted aortic arch; TOF, tetralogy of Fallot; PA, pulmonary valve atresia; PV, pulmonary valve; VSD, ventricular septal defect with malalignment of the conal septum; DGS/VCFS, DiGeorge/Velo-cardio-facial syndromes; M, maternal noncontribution; P, paternal noncontribution; NI, noninformative.

DISCUSSION

The availability of microsatellite DNA markers for detection of 22q11deletions has several practical consequences. First, the PCR-based genotypic analysis makes the experimental procedure fast (24 hours) and easy. Second, the high informativity of the marker makes detection of hemizygosity much easier as compared to RFLPs. Finally, loss of heterozygosity detected by these markers can be used as a reliable diagnostic tool in suspected cases of DGS/VCFS syndrome and in cono-truncal heart defects with associated minor anomalies as well. This test, which is currently performed in newborn infants referred to our institution for a conotruncal heart malformation, is now used as a first intention screening for 22q11 microdeletion. Also, it will probably reduce the indication of FISH to a limited number of cases as the PCR-based tecnique is particularly fast and cost-efficient. However, it should be borne in mind that a negative result does not rule out chromosome 22q11 deletions as some patients may prove to have smaller deletions or point mutations within essential gene(s) mapping to chromosome 22q11 [Budarf et al., 1995].

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